Gel Extraction

By Penn iGEM 2014

Goal: To extract DNA fragments from agarose gel, often used after PCR and digestion

Protocol

- Get an Eppendorf tube, and tare its weight
- Image Gel
- Cut the gel and put it in the tube.
- Weigh the gel and record its mass
  - only load max 400mg per column
- Add 3 times the mass of the gel of qG buffer (400mg=1200 uL)
- Incubate at 50C for 10mins - While waiting take out 2 or 3 times to vortex
- Add 1 times the mass of the gel amount of isopropanol to gel (300mg=300uL)
- Load 750 uL of that mixture into the gel extraction column (purple column)
- Spin down for 1 min at 13,000 rpm
  - Keep doing this(using the same column) until you harvest all the DNA in your sample
- wash with alcohol PE buffer to keep plasmid stuck on filter
  - check the cap sticker to make sure ethanol is addedadd 750 uL PE buffer to column filter
- centrifuge 13,000 RPM for 1 min, no liquid should be left on filter
- pour off Ethanol flow-through in collection tube into liquid waste evaporate alcohol by spin, centrifuge 13,000 RPM for 1 min
  - add Elution Buffer (EB: H20+ salts) to filter to elute DNA
- move filter over brand new Eppendorf 1.5mL tube
- see that it goes on to the filter, but do not puncture the filter
- add 35 uL EB to filter
- Let SIT ON BENCH on filter for 1 min